

# Cowpea N rhizodeposition and its below-ground transfer to a co-existing and to a subsequent millet crop on a sandy soil of the Sudano-Sahelian eco-zone

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**Abstract** Nitrogen (N) rhizodeposition by cowpea (*Vigna unguiculata* (L.) Walp) is potentially a large N source in cropping systems of Sub-Saharan Africa. A field experiment was conducted to measure cowpea N rhizodeposition under the conditions of the Sudano-Sahelian zone using direct  $^{15}\text{N}$  labelling techniques to trace the amount of deposition and its transfer to associated and subsequent crops. Half of the total cowpea crop N was located below-ground at plant maturity, which exceeded  $20 \text{ kgN ha}^{-1}$  when intercropped with millet. Only 15% of the below-ground cowpea N was recovered in roots, while 85% was found in the rhizodeposited pools. The experiment

demonstrated that direct below-ground N transfer occurred from cowpea to millet in intercrop at a rate of  $2 \text{ kgN ha}^{-1}$  over the growing season. Forty percent of the  $25 \text{ kg}$  below-ground N that the cowpea crop left at harvest were identifiable in the top  $0.30 \text{ m}$  soil in the beginning of the next planting season 7 months later; a pool still present at the end of that second season. Thus, the subsequent crop of millet (*Pennisetum glaucum* (L.) R. Br.) only recovered  $2.5 \text{ kgN ha}^{-1}$  from the below-ground cowpea pre-crop N during this growth season. The role and potential of cowpea as N provider has been underestimated in the past by ignoring the large proportion of N contained in its

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rhizodeposits. However, information is needed to determine how losses of the rhizodeposited N can be minimized to fully harness the potential of cowpea as N provider in agro-ecosystems of the region.

**Keywords**  $^{15}\text{N}$  · *Vigna unguiculata* · Leaf labelling · Legume · Nitrogen transfer · Tracer · Roots · Cowpea · *Pennisetum glaucum* · N deposition

## Introduction

Crop production in the Sudano-Sahelian eco-zone is limited by low soil nutrient availability, especially nitrogen (N) and phosphorus (P). Soils of the region are characterized by low organic C, total and available P and N, and effective cation exchange capacity (ECEC) (Bationo et al. 2002). Cowpea is the main legume grown in the region where it is cultivated for grain, fodder and for its soil improvement effects (Sanginga et al. 2003). The main cropping system of the region consists of an intercrop of millet and cowpea; 90% of the millet fields in Niger are intercropped with cowpea (Bationo et al. 2004).

Cowpea is thought to improve soil fertility by supplying N to the soil via symbiotic nitrogen fixation (Alvey et al. 2001; Bado et al. 2006). Knowledge of cowpea symbiotic nitrogen fixation and inputs to soil is based solely on measurement of above-ground plant parts (Bado et al. 2006; Bagayoko et al. 1998). Despite the fact that forage shoot material is mostly removed from the field to feed livestock in the Sudano-Sahelian region (Bationo et al. 2004; Singh et al. 2003), improved cereal yields following cowpea monoculture or cowpea/millet mixtures in the rotation have been reported (Alvey et al. 2001; Bado et al. 2006; Bagayoko et al. 1996, 1998; Fussell and Serafini 1985; Samake et al. 2006). It is therefore necessary to measure the N contained in the roots and rhizodeposits of cowpea to better understand the N effects in rotation and in intercrop with cereals (Bationo et al. 2004).

Rhizodeposits form a large part of legume below-ground N and include roots exudates, fine roots and root necrosis products accrued in the soil during plant growth (Hertenberger and Wanek 2004). Fine roots, exudates and decomposed material are not recovered when roots excavation is performed. Physical recovery of roots thus substantially underestimates the role of

the plants below-ground organs in nutrient cycling. Direct labelling methods using the stable isotope  $^{15}\text{N}$  have been developed and used to quantify N of plant origin and trace its fate in the soil (Khan et al. 2003; McNeill et al. 1997; Yasmin et al. 2006).

Studies carried out in the Guinean savannah of Nigeria indicated that below-ground N contribution from soybean is potentially a large source of N in agro-ecosystems of that region (Franke et al. 2008; Laberge et al. 2009). Similar findings were reported for legumes of temperate and Mediterranean regions (Gylfadóttir et al. 2007; Høgh-Jensen and Schjoerring 2001; Khan et al. 2003). To our knowledge, no information is available on rhizodeposition of cowpea and its potential transfer of N to millet in intercrop and rotation.

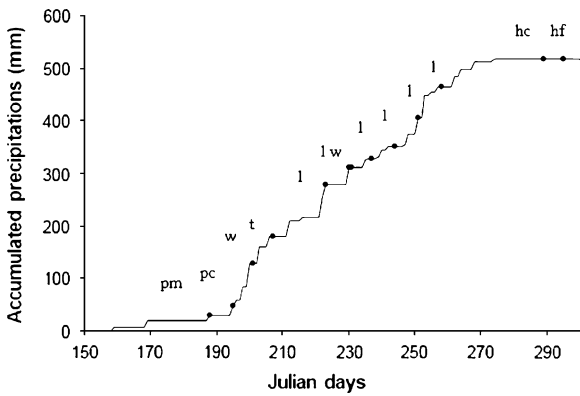
The objectives of this study were 1) to measure the contribution of rhizosphere processes to N fluxes during and following cowpea cultivation in the Sudano-Sahelian zone and 2) to measure below-ground N transfer to a millet crop grown in association or in rotation with cowpea. A third objective was to trace the fate of N accrued in cowpea roots and rhizodeposition in soil over a 1-year period. The hypotheses behind this study are 1) that N rhizodeposited by grain legumes is a substantial but hitherto ignored source of N in cropping systems of the Sudano-Sahelian zone, 2) that cowpea below-ground N contributes positively to the N supply of an associated or subsequent millet crop, and 3) that cowpea-derived below-ground N pools are highly degradable with a high potential to be taken up by plants or to be lost from the soil.

## Materials and methods

### Experimental site

The experiment was conducted in 2006 and 2007 at the ICRISAT Sahelian centre (ISC) in Sadore, Niger. The region is part of the Sudano-Sahelian agro-ecological zone, which is characterized by a mono-modal rainfall distribution (FAO 2004). Long-term (1983 to 2004) average annual precipitation at Sadore station was 542 mm. Soils at the experimental sites are arenosols (FAO 1988).

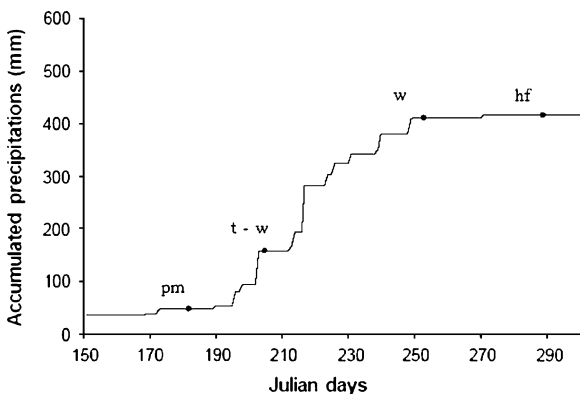
Rainfall, measured by a gauge at the experimental site, was close to the seasonal average in 2006 despite the season starting late (Fig. 1). Millet and cowpea



**Fig. 1** Accumulated precipitation in the growing season of 2006. The *dots* represent the different field activities. pm: planting millet, pc: planting cowpea, w: weeding, t: thinning, l: labelling, hc: harvest and sampling of labeled cowpea plots, hf: harvest of the entire field. The cowpea beans were harvested throughout the season

were planted late in the second and third weeks of July, respectively. The season also started late in 2007 with precipitation of 415 mm well below the seasonal average of 542 mm (Fig. 2). The rainfall events were well distributed over the season and millet yield at the experimental site was little affected by the rain deficit in 2007.

The site had been under natural fallow for 4 years when the experiment began. The field was disc-harrowed once prior to planting in 2006. Soil preparation was finished by hand and the plant residues were removed from the field. Soil samples were taken to a depth of 0–0.30 m before planting in 2005 using a soil auger 0.10 m in diameter. Twelve



**Fig. 2** Accumulated precipitation in the growing season of 2007. The *dots* represent the different field activities. pm: planting millet, p: planting cowpea, t: thinning w: weeding, hf: harvesting the field

samples were randomly taken over the field (1,740 m<sup>2</sup>) and combined to one composite sample for analyses. Soil particle size, pH (H<sub>2</sub>O, 1:2.5 soil to water ratio), organic C content (Walkley-Black method), total N (Macro-Kjeldahl method) and Bray-I extractable P (Table 1) were determined at ICRISAT Sadore’s analytical service lab. Soils of the experimental site are considered fertile with Bray-I extractable P values of 10 mg P kg<sup>-1</sup> in comparison to most soils of South-West Niger with Bray-I extractable P values below 3 mg P kg<sup>-1</sup> soil (Voortman et al. 2004).

Experimental set-up in 2006

Millet planted on 18th June 2006 failed to establish due to recurring drought periods and was replanted the 7th of July. Cowpea was planted 1 week later to minimize cowpea competition with millet following the normal practice in the area.

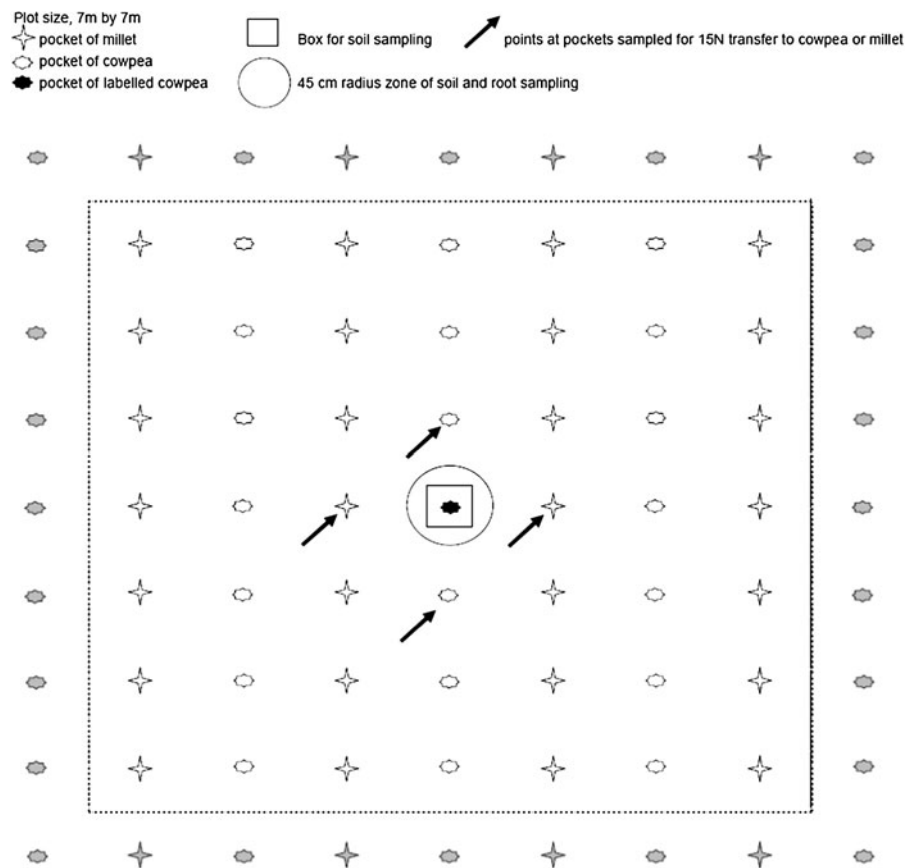
One row of millet planting holes, pockets, was alternated with one row of cowpea pockets throughout the field (Fig. 3). Cowpea and millet were planted in a regular grid pattern with 1 m between every pocket in a flat seed bed as is common practice in Western Niger. Fertilizer was applied at a rate of 1.1 g NH<sub>4</sub><sup>+</sup> and 0.9 g P<sub>2</sub>O<sub>5</sub> per pocket at planting, a fertilization strategy known as micro-dosing (Hayashi et al. 2007). The plants were thinned to three millet plants and two cowpea plants per pocket 3 weeks after millet planting. The timeframe for the field and sampling operations are indicated in Fig. 1.

The millet variety used in this experiment was the improved cultivar IT-89 305, maturing in 95 days. The cowpea variety was the Kodé local variety, collected in the village of Kodé close to Niamey. It is an indeterminate creeping variety grown for its high production of residues.

**Table 1** Characteristics of top 0.30 m soil at the experimental site in Sadore, Niger

Soil parameters	
pH (H <sub>2</sub> O)	4.8
Org. C%	0.25
Total N mg kg <sup>-1</sup>	155
Bray-I P mg kg <sup>-1</sup>	10.2
Sand%	95.3
Silt%	1.8
Clay%	2.9

**Fig. 3** Diagram of a plot where  $^{15}\text{N}$  labelling of cowpea was carried



The field was divided into 12 plots of each 7×7 m. The four plots at the four corners of the field were used to measure cowpea N fixation by the  $^{15}\text{N}$  isotope dilution method (IAEA 2004). The eight other plots (Fig. 3) were used to measure cowpea N rhizodeposition and cowpea N transfer to the associated millet crop using direct  $^{15}\text{N}$  labelling techniques (McNeill et al. 1997; Khan et al. 2003). Hand weeding was done 2 weeks and 8 weeks after millet planting. Crops were sprayed with an insecticide (Karate®, lambda-cyhalothrine, 150 ml a.i. ha<sup>-1</sup>) 3 and 9 weeks after millet planting.

Throughout 2006, shedded leaves and beans from the labelled cowpeas were collected in a tightly woven mosquito net fabric placed close to the ground around the labelled cowpea pockets. The captured leaves were removed frequently to avoid any enriched leaf material transferring into the soil.

#### Procedures for direct $^{15}\text{N}$ labelling of cowpea

The central pockets of eight plots were labelled and used for the rhizodeposition study. A multiple direct

$^{15}\text{N}$  labelling strategy was used to enrich simultaneously the cowpea plants that these central pockets contained. The two cowpea plants were enriched in  $^{15}\text{N}$  by weekly feeding them each 1 ml of 0.5%  $^{15}\text{N}$  urea (99.6 atom%  $^{15}\text{N}$ ) over 6 weeks. The procedures for labelling the plants followed the leaf-flap methods described by Khan et al. (2002): a V-shape leaf-flap was cut underwater using a scissor with the tip of the V centred on the mid-vein of one leaflet. The leaflet-flap was then rapidly inserted in a 5-ml plastic vial containing 1 ml of the urea solution. The vial was sealed using inert plastic material after ensuring that the leaf flap was in contact with the solution. To maintain the vial in position it was attached to a short wooden stick anchored in the soil. Leaflets chosen for labelling were selected from the lower part of the stem for ease of labelling operations.

The vials were left in place for 24 h to 48 h depending on the speed of solution uptake and were then removed. The labelled leaflets were removed with the vials after labelling to avoid contaminating the soil with  $^{15}\text{N}$  that might have remained in the

leaflets. Labelling started on the fifth week after planting millet; the cowpea plants were 1-month old. The plants were all then pulse labelled once a week for 6 weeks.

#### Procedures for $^{15}\text{N}$ of soil N pools to determine symbiotic nitrogen fixation

Symbiotic nitrogen fixation in the cowpea was measured using the  $^{15}\text{N}$  isotope dilution method (IAEA 2004) with millet used as a non-fixing reference plant. Four plots ( $7 \times 7$  m) were kept solely to measure  $\text{N}_2$ -fixation. The same planting pattern was used as in the rest of the field but millet was planted on the same date as the cowpea. A solution of  $\text{KNO}_3$  (5.6 g, 98 atom%  $^{15}\text{N}$  in 20 L of water) was uniformly applied over an  $8 \text{ m}^2$  area in every plot with a watering can. Sucrose (18 g) was added to the solution to obtain a C: N ratio of approximately 10:1 leading to an initial immobilization of the  $^{15}\text{N}$  and to have a relatively constant enrichment of the plant available N throughout the season (Giller and Witty 1987; Witty and Roose 1984).

#### Dry matter measurement of millet and cowpea at maturity

The dry matter of cowpea, beans and residues and the dry matter of millet, grain and residues were measured by harvesting three areas ( $15 \times 15$  m) at different locations throughout the field. Cowpea beans were harvested throughout the season when the pods were maturing. The harvested material was air dried in a greenhouse to determine its dry matter content. The final harvest of cowpea plants and millet took place on the 22<sup>nd</sup> of October.

#### Sampling for $^{15}\text{N}$ determination

The whole above-ground direct  $^{15}\text{N}$  labelled cowpea plants were harvested on the 16th of October. One neighbouring pocket of millet was harvested to measure N transfer from cowpea to millet. One neighbouring pocket of cowpea was also harvested to measure N transfer from cowpea to cowpea. Soil and roots were also sampled on that date. Weeds growing within 0.45 m of the labelled cowpeas were collected throughout the season also to measure cowpea N transfer to weeds.

Sampling of soil and roots under and around the labelled cowpea plants took place in a two-step manner. First, to sample what is termed the 'pockets soil', a metal box ( $0.30 \text{ m} \times 0.30 \text{ m} \times 0.15 \text{ m}$  depth) was inserted in the soil precisely over the pocket. Secondly, to sample what is considered the soil affected by the individual plants rooting, termed 'rooting soil', a circle with 0.45 m radius was traced on the soil with the box at its centre. All the soil and roots within this circle and outside of the box was dug out to a depth of 0.15 m and kept in large plastic pots. After the soil within the circle was removed, a metal flap was inserted under the box and the content of the box was transferred to a container. The whole procedure was repeated to sample between 0.15 m to 0.30 m depth.

The samples were dry-sieved through a 2-mm mesh and all visible roots collected. The sieved soil was thoroughly mixed and a 100 g sub-sample was taken and dried to constant weight at  $60^\circ\text{C}$  for 72 h to determine soil water content and soil dry weight. These soil samples were also the ones analysed for  $^{15}\text{N}$  content. Four soil-root samples were thus obtained around the labelled plants, two within the pocket soil at 0–0.15 m and 0.15–0.30 m depth, and two samples from the rooting soil, i.e. within the 0.45 m radius but outside the box at 0–0.15 m and 0.15–0.30 m depth (Fig. 3).

Five control cowpea plants and their root systems were sampled from the border rows to determine the natural  $^{15}\text{N}$  enrichment of their N pools. Five control samples of soil were also taken from the border at two different depths: 0–0.15 m and 0.15–0.30 m. Millet was harvested from five pockets in the borders to be used as control in the calculations of N transfer. Weeds growing in the border plots were also collected to determine their enrichment and use them as controls against enrichment of weeds collected close to the labelled cowpeas. Only the above-ground parts of the weeds were collected.

The  $^{15}\text{N}$  enriched area for symbiotic nitrogen fixation determination had at its centre a pocket with cowpea and two pockets of millet. These three pockets were harvested together at the end of the experiment to measure symbiotic nitrogen fixation. The whole millet and cowpea above-ground sections were sampled to determine their  $^{15}\text{N}$  enrichment.

### Experimental set-up in 2007 to determine pre-crop effect

All above-ground plant residues had been removed from the field in 2006. The soils sampled for determination of cowpea below-ground N in 2006 were returned to their respective plots along with the roots of cowpea 2 days after sampling in October 2006. Subsequently, new millet was established in the field in 2007 to estimate the N pre-crop effect.

Millet planted on 1st of June 2007 failed to establish in the absence of subsequent rainfall but a second planting on the 1st of July established readily. The plants were thinned to two plants per pocket on the 24th of July. The field was weeded on the 24th of July and on the 10th of September. The field was harvested and sampled on the 16th of October 2007. The timing of the field operations is presented in Fig. 2 along with the accumulated precipitation in 2007.

Pockets of millet were grown directly over the pockets where cowpea was labelled in 2006 to determine transfer of cowpea below-ground N to millet from 1 year to the next. The millet plants growing over the labelled cowpea were harvested at maturity and treated as the millet intercropped in 2006; see above, to determine their biomass, N content, and  $^{15}\text{N}$  enrichment.

### Decay patterns of below-ground cowpea N

A sub-experiment was initiated in the field alongside the main experiment in 2006. The objective of the sub-experiment was to follow the decay of cowpea below-ground N following cowpea harvest.

Twelve round free-draining plastic containers (0.35 m deep, 0.20 m radius) were placed 0.30 m deep in the soil, leaving the edge of them 5 cm above the soil surface. Once in place, the containers were filled with the soil displaced during their insertion. The soil was sieved to remove the large pieces of organic material and facilitate root sieving in the later stages of the experiment. Two cowpea plants were established in every container and these plants were treated and labelled in exactly the same way and at the same time as the cowpea grown in the field in the labelled plot in 2006; plants from two containers were unlabelled as control. The

sampling followed the same procedures as the sampling in the field.

Following sampling, the roots and soil were returned to the containers in the field. The containers were left in place without plants and were sampled twice more to determine their soil  $^{15}\text{N}$  enrichment in June 2007 at planting of millet and in October 2007 after harvest of the millet crop. The soil samples were taken directly under the millet pocket of 2007, where cowpea had been grown in 2006 to trace the fate of the  $^{15}\text{N}$  labelled legume below-ground N.

### $^{15}\text{N}$ determination of plant and soil samples

Grain, beans, residues, roots and weeds were dried to constant weight in a forced air oven at  $60^\circ\text{C}$ , for 72 h weighed and ground with a rotary mill (Wiley Mill, A.H., Thomas, Philadelphia, PE, USA) to pass a 0.5 mm sieve. Subsequently, soils, pods, residues and roots samples were analyzed for total N content and  $^{15}\text{N}$  enrichment by flash combustion on an elemental analyzer (EA1110, Carlo Erba, Milan, Italy) coupled in continuous flow mode with an isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany).

Calculations of  $^{15}\text{N}$  partitioning, rhizodeposition, transfer to intercropped millet and weeds and symbiotic nitrogen fixation

$^{15}\text{N}$  enrichment is expressed as  $\delta^{15}\text{N}$  (‰) relative to the natural enrichment of atmospheric N (0.3663 atom‰) as in Eq. 1.

$$\delta^{15}\text{N} = \frac{\left( \text{ratio}^{15}\text{N}/^{14}\text{N}_{\text{sample}} - \text{ratio}^{15}\text{N}/^{14}\text{N}_{\text{atmosphere}} \right)}{\text{ratio}^{15}\text{N}/^{14}\text{N}_{\text{atmosphere}}} \times 1000 \quad (1)$$

The proportion of soil N derived from rhizodeposition (%N<sub>dfr</sub>) was calculated from the ratio of enrichment of labelled soil within a given sampled volume over that of labelled roots within the same sampled volume. In the calculations, the enrichment of the labelled soil and roots was corrected against the

enrichment of the same N pool in the controls (Schmidtke 2005) as in Eq. 2.

$$\% Ndf_r = \frac{\delta^{15}\text{N Soil Labelled} - \delta^{15}\text{N Soil Control}}{\delta^{15}\text{N Root Labelled} - \delta^{15}\text{N Root Control}} \times 100\% \quad (2)$$

The quantity of soil N coming from rhizodeposition was obtained by multiplying the total soil N with %Ndf<sub>r</sub>. Values obtained for below-ground measurements around the pocket of the labelled cowpea were converted to per area unit basis by multiplying them by the ratio of the above-ground biomass of cowpea measured in the whole field over the biomass measured in the labelled pocket.

Nitrogen transfer from the labelled cowpea plants to the two neighbouring cowpea plants, the two neighbouring millet plants and weeds was measured by comparing <sup>15</sup>N enrichment in the cowpea roots and in the receivers above-ground biomass. The average enrichment of the donor cowpea roots in the rooting soil, i.e. the 45 cm radius to a depth of 0.30 m, were used in this calculation. The proportion of N in the receiver plant was calculated using Eq. 3 as given by Giller et al. (1991).

$$\% Ntransfer = \frac{\delta^{15}\text{N Receiver} - \delta^{15}\text{N Control}}{\delta^{15}\text{N Roots} - \delta^{15}\text{N Root Control}} \times 100\% \quad (3)$$

The quantity of N transferred to the two neighbouring millet plants was obtained by multiplying the total receiver N with %N transfer.

To determine symbiotic nitrogen fixation, the <sup>15</sup>N enrichment of the whole cowpea above-ground parts was compared to that of millet above-ground parts. % Ndfa was calculated by using Eq. 4.

$$\% Ndfa = \frac{(\delta^{15}\text{N millet} - \delta^{15}\text{N cowpea})}{\delta^{15}\text{N millet}} \times 100 \quad (4)$$

The amount of N fixed by cowpea was determined using this value multiplied by total N accumulated in the cowpea crop and its rhizodeposits.

The pre-crop N in millet plants derived from the previous cowpea plants was determined using Eq. 5, where the <sup>15</sup>N enrichment of cowpea below-ground

N is the average enrichment of the rhizodeposits and roots in the soil under the labelled pocket. The equation is adapted from previous equations on labelled fertilizer uptake (IAEA 2004).

$$\% Ndf \text{ cowpea BGN} = \frac{(\delta^{15}\text{N millet} - \delta^{15}\text{N millet control})}{(\delta^{15}\text{N of cowpea BGN})} \times 100 \quad (5)$$

The amount of N transferred to the subsequent crop was obtained by multiplying the millet N content with %Ndf cowpea BGN.

#### Data handling

Average biomass, N content and <sup>15</sup>N content were calculated from the measurements taken from the eight labelled pockets. Results were presented with their corresponding mean standard errors. The same was done for pockets of millet and cowpea and for weeds with the data on transfer. Two-ways ANOVA were carried out using R (R Development Core Team 2007) with the replicates and organs as criteria to test whether the labelled cowpea plants were uniformly enriched in <sup>15</sup>N following labelling.

## Results

Millet and cowpea biomass, N content and cowpea symbiotic nitrogen fixation

Millet intercropped with cowpea in this experiment yielded above average for the region with a total biomass accumulation of 3.4 Mg of DM ha<sup>-1</sup> of which 1.4 Mg of DM ha<sup>-1</sup> was in the spikes. The millet crop contained 50 kgN ha<sup>-1</sup> of which 30 kgN ha<sup>-1</sup> was located in the grain (Table 2).

**Table 2** Millet dry matter on a per area unit basis at harvest in Sadore, Niger, in 2006 and 2007. Mean ± SE (n=6)

	2006 kg DM ha <sup>-1</sup>	2007 kg N ha <sup>-1</sup>
Spikes	1432±149	1757±219
Leaves	493±62	630±133
Stems	1450±164	1553±159

Cowpea accumulated 1.0 Mg DM ha<sup>-1</sup> in its above-ground organs of which 0.8 Mg DM ha<sup>-1</sup> was in the harvested residues, the balance being in the grain and shedded leaves (Table 3). The low harvest index is attributed to the indeterminate growth of this variety and to insect attacks on flowers, mainly by *Mylabris spp.* The above-ground sections of cowpea accumulated 23 kgN ha<sup>-1</sup> (Table 4).

Recoverable cowpea roots biomass in the rooting soil amounted to almost 300 kg DM ha<sup>-1</sup> (Table 3). Approx. 30% of the root dry matter was located in the central 0.09 m<sup>2</sup> core around the planting pocket soil and 90% was located in the upper 0.15 m soil layer of the rooting zone. The recoverable roots of cowpea contained 3 kgN ha<sup>-1</sup> (Table 4). Cowpea derived on average 55% of its N from the atmosphere (data not shown).

#### Cowpea and soil <sup>15</sup>N enrichment after leaf-labelling

Cowpea roots and the soil in their surroundings were sufficiently <sup>15</sup>N enriched relatively to the controls to measure cowpea rhizodeposition. The <sup>15</sup>N enrichment

varied amongst the cowpea organs after labelling although this difference was not significant ( $P=0.08$ ). The <sup>15</sup>N enrichment of the roots recovered in the rooting soil in the 0.15–0.30 m depth had lower enrichment than the roots closer to the surface or than the roots from the pocket soil (Table 3). The soil was similarly enriched in the samples taken within the 0.45 m radius of the pocket and within the pocket soil in the lower depth from 0.15–0.30 m. Soil <sup>15</sup>N enrichment was higher in the pocket soil at 0–0.15 m depth compared to the other sampling locations, there were also more roots in the top 0–0.15 m depth (Table 3).

#### Cowpea rhizodeposition

More than half of the total cowpea N was located below-ground in roots and rhizodeposits. More than 85% of this below-ground cowpea derived N was found in the rhizodeposits. 72% of the N in roots and rhizodeposits were located in the top 0.15 m of soil. The rhizodeposits of the cowpea contained 21.5 kgN ha<sup>-1</sup> (Table 4).

**Table 3** Cowpea dry matter at harvest on a per area unit basis and cowpea <sup>15</sup>N enrichment of both controls and labelled plants in Sadore, Niger, in 2006. Mean ± SE ( $n=6$ )

	Cowpea biomass (kg DM ha <sup>-1</sup> )	<sup>15</sup> N enrichment	
		δ <sup>15</sup> N control	δ <sup>15</sup> N labelled
Above-ground			
Grain	126±30	1.5±0.5	551±341
Residues	829±147	1.5±0.5	205±27
Shed leaves	60±9	1.5±0.5	731±108
Below-ground “pocket soil” within the box			
Roots/0–0.15 m	81±7	4.3±1.4	264±50
Roots/0.15–0.30 m	3±1	4.3±1.4	331±98
Soil/0–0.15 m	–	5.1±0.2	48±6
Soil/0.15–0.30 m	–	5.5±0.6	18±1
Below-ground ‘rooting soil’ radius			
Roots/0–0.15 m	169±17	4.3±1.4	159±12
Roots/0.15–0.30 m	24±7	4.3±1.4	77±22
Soil/0–0.15 m	–	5.1±0.2	15±2
Soil/0.15–0.30 m	–	5.5±0.6	17±2
Total			
Above-ground total	1015±179		
Below-ground total	278±23		
Total	1293±187		



**Table 4** Nitrogen in cowpea and cowpea rhizodeposits in Sadore, Niger in 2006. Mean  $\pm$  SE ( $n=6$ )

Cowpea N	mg N pocket <sup>-1</sup>	kg N ha <sup>-1</sup>	% of total N
Aboveground			
Grain	902 $\pm$ 234	4.3 $\pm$ 1.1	8.3 $\pm$ 1.5
Residues	3835 $\pm$ 774	18.2 $\pm$ 3.7	36.3 $\pm$ 2.9
Shed leaves	218 $\pm$ 34	1.0 $\pm$ 0.2	2.1 $\pm$ 0.3
Below-ground “pocket soil”			
Roots/0–0.15 m	248 $\pm$ 26	1.2 $\pm$ 0.1	2.5 $\pm$ 0.2
Roots/0.15–0.30 m	10 $\pm$ 3	0.05 $\pm$ 0.01	0.1 $\pm$ 0.01
Soil/0–0.15 m	1035 $\pm$ 134	4.9 $\pm$ 0.6	10.2 $\pm$ 0.6
Soil/0.15–0.30 m	168 $\pm$ 44	0.8 $\pm$ 0.2	1.5 $\pm$ 0.3
Below-ground “rooting zone”			
Roots/0–0.15 m	407 $\pm$ 59	1.9 $\pm$ 0.3	4.0 $\pm$ 0.3
Roots/0.15–0.30 m	59 $\pm$ 18	0.3 $\pm$ 0.1	0.6 $\pm$ 3.2
Soil/0–0.15 m	2189 $\pm$ 107	10.4 $\pm$ 0.5	22.7 $\pm$ 2.1
Soil/0.15–0.30 m	1143 $\pm$ 118	5.4 $\pm$ 0.6	11.6 $\pm$ 1.1
Above-ground total	4955 $\pm$ 1006	23.5 $\pm$ 4.8	47 $\pm$ 3
Below-ground total	5259 $\pm$ 434	25.0 $\pm$ 5.1	53 $\pm$ 3
Total	10214 $\pm$ 1320	48.5 $\pm$ 6.3	100 $\pm$ –

N transfer from labelled cowpea to millet, cowpea and weeds

Millet plants grown in proximity of the labelled cowpea had significantly higher <sup>15</sup>N enrichment than the millet used as control (Table 5). On the other hand, the unlabelled cowpea plants growing in proximity of the labelled cowpea had similar enrichment to cowpea used as control, which implies that cowpea to cowpea N transfer is negligible and that

millet is the main competitor for cowpea derived N from rhizodeposition.

The N in millet that was derived from cowpea was 2.7 and 4.4% of the N contained in spikes and leaves, respectively, which correspond to a net transfer of 1.4 and 0.7 kg N ha<sup>-1</sup>. Assuming a similar proportion of N transferred in the stems and roots would add approx. 1 kg from stems and 2 kg N from roots to the balance, reaching an amount transferred of approx. 5 kg N ha<sup>-1</sup> (Table 5).

**Table 5** <sup>15</sup>N enrichment and N content of millet, cowpea and weedy plants growing beside the labelled cowpea in 2006 and N transferred from labelled cowpea to these different receivers through belowground N transfer, mean  $\pm$  SE ( $n=6$ )

	<sup>15</sup> N enrichment		N content in neighbouring millets		N transferred from cowpea	
	$\delta^{15}\text{N}$	$\delta^{15}\text{N}$ of controls	(mg N pocket <sup>-1</sup> )	(kg N ha <sup>-1</sup> )	(%) <sup>a</sup>	(kg N ha <sup>-1</sup> ) <sup>b</sup>
Millet						
Spike	8.4 $\pm$ 1.2	4.1 $\pm$ 0.3	6022 $\pm$ 752	28.6 $\pm$ 3.6	2.7 $\pm$ 0.7	1.4 $\pm$ 0.3
Leaves	10.9 $\pm$ 0.7	4.1 $\pm$ 0.3	1740 $\pm$ 264	8.3 $\pm$ 1.3	4.4 $\pm$ 0.7	0.7 $\pm$ 0.1
Stem	– $\pm$ –	– $\pm$ –	2537 $\pm$ 332	12.1 $\pm$ 1.6	NA $\pm$ NA	NA $\pm$ NA
Cowpea-residues	1.8 $\pm$ 0.3	1.5 $\pm$ 0.3	2462 $\pm$ 874		0.3 $\pm$ 0.2	
Weeds	29.0 $\pm$ 10.8	4.1 $\pm$ 0.3	49 $\pm$ 25		18.5 $\pm$ 8.0	

<sup>a</sup> The % represents the N transferred from one labeled cowpea to one millet plant growing beside it in our experiment

<sup>b</sup> The N transferred on a kg ha<sup>-1</sup> basis represents the N transferred from cowpea plants to the millet plants growing around them. In this case, it was assumed that the cowpea contributed N to both millet plants growing directly beside it, on each side of the legume at a 1 m distance

## Subsequent millet utilisation of pre-crop cowpea derived N

The pure stands of millet grown in the field in 2007 produced high yields for the area with a biomass production of 3.9 Mg DM ha<sup>-1</sup> and a grain production of 1.8 Mg DM ha<sup>-1</sup> (Table 2), which is equivalent to a total of 61.5 kgN ha<sup>-1</sup> (Table 6). Millet directly derived 4.3% of its N from the pre-crop N pools located below-ground of the cowpea grown in the same pocket the preceding growing season. This proportion equals 2.6 kgN ha<sup>-1</sup> (Table 6).

The fate of cowpea derived N from cowpea harvest to subsequent millet crop seeding was monitored in the sub-experiment using soil cores. In the beginning of the planting season, June 2007, 40% of the excess <sup>15</sup>N present in October 2006 remained in the pots. Sampling in June was done a few weeks after the first rain. By harvest time, October 2007, 1 year after the harvest of cowpea, 36% of the excess <sup>15</sup>N from the rhizodeposits and roots remained in the pots (Table 7) with two-thirds still located in the top 0.15 m soil. As the sampled volume in the field study corresponded to the section of soil where samples were taken within the top 0.15 m of the soil cores, these figures strongly indicate that the residue N and the N deposited does not significantly move vertically through the soil profile. Losses may thus mainly occur during the dry season and the pools recoverable at the onset of the growth season appear relatively recalcitrant.

## Discussion

The yield of millet in 2006 with 1.4 Mg DM ha<sup>-1</sup> of grain were high compared to general yields of less than 1 Mg DM ha<sup>-1</sup> common to the region (Table 2) (Voortman et al. 2004; Gandah et al. 2003; Fofana et

al. 2008). This productivity reflected the high soil fertility at the site, including the long fallow period and the application of P using the micro-dose technique (Tabo et al. 2005; Hayashi et al. 2007). The cowpea variety also accumulated higher biomasses, 1 Mg DM ha<sup>-1</sup>, compared to typical yields of less than 0.4 Mg DM ha<sup>-1</sup> in intercrop in the area (Table 3; Biielders et al. 2000). The results of this study thus represent the potential N contribution of cowpea to the farming systems of the Sudano-Sahelian eco-zone.

This is the first time that legume rhizodeposition and its subsequent fate has been quantified in the field using a <sup>15</sup>N direct labelling techniques without constraining the roots of the plants (e.g. Høgh-Jensen and Schjoerring 2001; Khan et al. 2003). It is demonstrated that it is feasible to measure N rhizodeposition in this way as the roots and soils were sufficiently enriched (Table 3) to accurately measure N rhizodeposition, which is especially important when working with plants that develop large root systems.

The method used is based on the assumption that plant N pools will be equally labelled over time. This was attempted by labelling over 6 weeks during the period with the fastest growth. An incorporation of the tracer into the strongest sinks must be expected and this is also apparently the case in this study as shedded leaves and grains are more enriched than the largest N pool, i.e. residues. This may lead to an overestimation of rhizodeposition (Eq. 2). However, a weighted mean of the enrichment, calculated by weighing the enrichment by the N pool size of each organ, would be very close to the value of the enrichment of roots in the upper part of the pocket soil (Table 3), which underline the robustness of the method.

During the labelling, the leaf parts being directly labelled were removed to eliminate any potential

**Table 6** Millet <sup>15</sup>N enrichment, N content and N uptake from roots and rhizodeposited cowpea N in 2007, 1 year after the labelled cowpea was grown, Mean ± SE (n=6)

	<sup>15</sup> N enrichment		N content	Millet N derived from cowpea BGN	
	δ <sup>15</sup> N	δ <sup>15</sup> N-control	kg N ha <sup>-1</sup>	%	kg N ha <sup>-1</sup>
Millet					
Spikes	10.0±1.8	4.1±0.3	38.3±5.9	3.3±1.1	1.2±0.5
Leaves	15±1.1	4.1±0.3	8.8±1.5	6.2±1.7	0.6±0.1
Stems	15±0.5	4.1±0.3	14.4±2.0	5.9±1.1	0.9±0.1
Whole crop			61.5 7.1	4.3 0.9	2.6 0.7

**Table 7** Excess  $^{15}\text{N}$  in pots and in top 0.15 m of experimental plots (inside the box-sampling) remaining over the course of the experiment from October 2006 to October 2007

	Pots				Experimental plots-top 0.15 m inside the box			
	Excess $^{15}\text{N}$		% N remaining		Excess $^{15}\text{N}$		% N remaining	
	(mg) mean	SE	(%) mean	SE	(mg) mean	SE	(%) mean	SE
October-06	1.366± 0.109		100.00±7.95		1.085±0.122		100.00±11.22	
June 07	0.549± 0.112		40.23±8.18					
October 07	0.494± 0.114		36.19±8.35		0.257±0.069		23.70±6.39	

contamination from the source. Shedded leaves were captured in a fine woven net and they are not expected to be any source of error due to the minor amount of N that may have entered the soil this way.

#### Transfer of N from cowpea to millet, cowpea and weeds

The current labelling technique has been used in the field to study N transfer between closely associated perennial forage species (Gylfadóttir et al. 2007; Høgh-Jensen and Schjoerring 2001; Høgh-Jensen et al. 2006) but our study is the first occasion in which it is taken to full field conditions to study N transfer between more distantly grown grain legumes and cereals. It was demonstrated that transfer contributes modestly to the overall N economy of the receiving crop; including root N it amounted to approx. 5 kgN ha<sup>-1</sup> (Table 5), which must be seen in relation to the 50 kgN in the millet crop.

The high proportion of cowpea derived N in weeds growing closer to the donor plants and the modest proportion of transfer to neighbouring millet plants indicate that the plant-available cowpea derived N pools are available. However, they may largely be re-assimilated by the donor as a distance of 1 m from the receiver plants neighbouring the donor gives poor opportunities to access the cowpea derived N pools. Further, the delayed planting of cowpeas in a medium duration crop like the current, maturing in 95 days, may leave a relatively short time window for the transfer to take place. Consequently, values of 15% transfer from beans to maize obtained by Giller et al.

(1991) in a greenhouse experiment may overestimate field conditions.

#### Pre-crop N transfer from intercropped cowpea to subsequent pure stand millet

One hypothesis tested in the current study was that rhizodeposition of legume N is potentially a large N input in the farming systems of the Sudano-Sahelian eco-zone. The current study confirmed this hypothesis as 50% of the total accumulated cowpea N was located below-ground, more than three quarters of which was found in the physically unrecoverable rhizodeposited fractions (Table 4). A review of grain legumes N rhizodeposition studies recently indicated that, on average, 33% of grain legumes N are found below-ground (Wichern et al. 2008) but this will obviously depend on growth conditions and legume species. Our findings indicate that previous studies quantifying the N contribution by cowpea in various cropping systems underestimate its N contribution by measuring only its above-ground N content (e.g. Eaglesham et al. 1982; Bado et al. 2006) or by underestimating its below-ground N contribution (e.g. Adjei-Nsiah et al. 2008).

Up to 95% of the rhizodeposited N was located in the soil top layer from 0–0.15 m (Table 4) which agrees with other studies (Gylfadóttir et al. 2007; Høgh-Jensen and Schjoerring 2001; Høgh-Jensen et al. 2006; Khan et al. 2003; Araujo et al. 2006).

Generally a cowpea pre-crop will increase the yield of subsequent millet crops compared to continuous millet (Bagayoko et al. 1998; Alvey et al. 2001;

Samake et al. 2006) caused most likely by an improved soil N availability (Bagayoko et al. 2000; Shahandeh et al. 2004; Bado et al. 2006) and to an N sparing effect from growing N<sub>2</sub>-fixing legume crops (Giller 2002). Our results demonstrate that the uptake of cowpea derived below-ground N by a subsequent millet crop the following growth season was relatively small with a N uptake of less than 3 kgN ha, equivalent to around 5% of the total N uptake by millet.

The present study took place on a sandy soil typical for the Sahelian eco-zone. The early rain showers may flush such soils and pre-crop N effects are consequently expected to be lower (Shahandeh et al. 2004). In corresponding studies, the cereal crop recovered 10% and 34% of the pre-crop soybeans below-ground N in Brazil (Araujo et al. 2006) and Tanzania (Vesterager et al. 2007), respectively. A pool substitution effect (Jenkinson et al. 1985) is unlikely to be important due to the very low levels of N pools in the soil (Table 1).

Fate of cowpea below-ground N during a 1 year period after harvest

A large proportion of the cowpea N residues measured in October 2006, i.e. at harvest, had disappeared from the top-soil by June 2007, i.e. after the dry season (Table 7). Plant rhizodeposits are to a large extent made of rapidly degradable N compounds that can easily be mineralized or immobilized by the soil microbial population (Merbach et al. 1999; Hertenberger and Wanek 2004). N rhizodeposits are thus considered a rapidly cycling N flow with a high potential for losses on the sandy soils of the experiment with low activity clay and low organic matter content. Disappearance of the <sup>15</sup>N from the experimental plots and pot experiment is likely to be the results of N leaching and/or volatilization.

The onset of the rains after a dry period brings a rapid N mineralization, followed by a decrease in the soil N mineralization rate during the rest of the rainy season (Bernhardt-Reversat 1982; Shahandeh et al. 2004). Substantial rainfall occurring prior to the establishment of a standing crop holds the potential for soil N losses. Measurements of the soil <sup>15</sup>N content in June 2007 were taken after a few heavy showers and nitrate-N may already have been leached

from the top soil layers. Low N use efficiencies have been reported under eastern African condition where heavy rain showers may leach N below the rooting zone (Semb and Robinson 1969; Vesterager et al. 2008).

Soil <sup>15</sup>N content decreased comparatively little during the rainy season from June to October 2007 with the greater share of the most degradable pool of rhizodeposited N having already been lost from the system (Tables 5 and 6). The losses may be ascribed to a phenomenon known as the “Birch effect” (Birch 1964) where drying and rewetting of the soil as occurs at the beginning of the rainy season produces a disproportionately large and uniform release of C and N.

Utilisation efficiency of the pre-crop cowpea below-ground N by the first subsequent millet crop was small compared to N losses and the N remaining in the soil. More information is needed on how these N pools losses can be managed in the Sudano-Sahelian regions in order to grasp the full potential of N fixation by grain legumes.

## Conclusion

Below-ground plant derived N was found to constitute 50% of the total cowpea N in intercrop in 2006. The rhizodeposited N pools were eight times larger than the N pool in the recoverable roots. Below-ground N transfer from cowpea to millet in intercrop was demonstrated under field conditions at significant levels of approx. 10 kgN ha<sup>-1</sup>. Most of the cowpea below-ground N had disappeared from the top-soil before planting of millet in 2007. Forty percent of the excess <sup>15</sup>N remained in the soil in June 2007. The transfer from below-ground N of cowpea to millet in rotation was 5 kgN ha<sup>-1</sup>. The experiment showed that cowpea rhizodeposition is a potentially large input of N in cropping systems of the Sudano-Sahelian region.

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